Using trophic structure to reveal patterns of trait-based community assembly across niche dimensions

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Summary

1. Trait-based approaches for studying community assembly have improved understanding of mechanisms; however, the challenge of interpreting process from pattern is complicated by the possibility of multiple mechanisms operating simultaneously. Different traits may influence the assembly process in different ways. Analysing patterns of functional diversity among co-occurring species for each trait individually may aid interpretation of complex assembly processes; yet, few studies have tested whether patterns vary depending on trait function.

2. We used tropical fish assemblages from the Xingu River, Brazil to test whether traits associated with resource acquisition play a stronger role in niche segregation relative to other traits. First, a null modelling approach was used to determine how trait distributions within local assemblages deviated from expectations under random assembly. Then, correlations between functional traits and stable isotope ratios (δ^{13}C, δ^{15}N) were used as a measure of a trait's association with trophic structure. Finally, we used mixed effects models to test whether traits having higher correlations with trophic structure also had greater deviation from null expectations. In addition, we explored how well stable isotopes explain multivariate functional trait variation and compared our correlation-based approach for organizing traits with previous categorical approaches.

3. A significant relationship was found between a trait's deviation from null expectations and its correlation with isotopic patterns. Traits strongly associated with trophic structure had greater dispersion from the assemblage mean and were more evenly spaced than weakly associated traits. Traits strongly associated with trophic structure also were more clustered because trophic diversification tended to occur around some basic feeding strategies, such as benthic grazing or capturing food items from the water column. Based on redundancy analysis, isotopic ratios explained a low (11·6%) but significant portion of trait variation.

4. In this study system, traits strongly associated with trophic ecology were more influential in niche differentiation among coexisting species compared to weakly associated traits. These results suggest that certain traits may respond to assembly mechanisms in predictable ways despite the complex, multidimensional nature of the assembly process. Methods designed to identify differential trait response will be critical to developing a general theory of trait-based community assembly.

Key-words: α-niche, community assembly, fish, functional diversity, null model, stable isotope, Xingu River

Introduction

Trait-based approaches offer a path towards greater generality and predictability for community ecology (McGill *et al*. 2006), and a growing body of research evaluates patterns of functional diversity to infer mechanisms of community assembly (Weiher *et al*. 2011; Gotzenberger *et al*. 2012). Patterns of functional diversity of co-occurring species can be compared to that expected under random assembly to test whether communities are structured by

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mechanisms promoting functional similarity (e.g. environmental filtering, competitive hierarchies) or functional divergence (e.g. limiting similarity). The challenge of interpreting process from pattern, however, is complicated by the potential for both types of mechanisms to operate simultaneously within an assemblage, thereby obscuring expected patterns (Weiher, Clarke & Keddy 1998; Cavender-Bares *et al*. 2004; Grime 2006; Kraft, Valencia & Ackerly 2008; Ingram & Shurin 2009; Swenson & Enquist 2009). For example alpine plant communities in resource-poor, wind-exposed environments are functionally similar in certain above-ground traits (plant height, specific leaf area) due to abiotic filtering, but functionally divergent in traits associated with resource uptake (stomatal conductance, chlorophyll content) due to below-ground competition (Spasojevic & Suding 2012). Because these processes influence functional diversity in opposite ways, multivariate measures of functional diversity (i.e. the combined effect of all traits) showed no change relative to plant communities in resource-rich areas. In addition, it is becoming increasingly clear that similar distributions of functional traits within local assemblages may result from different mechanisms of community assembly (HilleRisLambers *et al*. 2012; Herben & Goldberg 2014; Kraft *et al*. 2014), making multivariate patterns difficult to interpret.

Recent theoretical and empirical studies have emphasized the need to analyse functional diversity based on individual traits or groups of traits related to specific functions to fully understand how trait distributions within communities reflect underlying assembly mechanisms (Spasojevic & Suding 2012; Laughlin 2014; Trisos, Petchey & Tobias 2014; Troia & Gido 2015; Winemiller *et al*. 2015). For example while functional divergence in traits associated with resource acquisition may imply a strong influence of competition, functional divergence in traits associated with life-history strategies may reflect different mechanisms, such as response to predation pressure or strategies to cope with variable environmental conditions (Grime 2006). Niches have multiple dimensions, and functional diversity may deviate from random expectations in different ways depending on the traits analysed. The degree of consistency in an individual trait's response to a given mechanism will ultimately determine the predictive power of the trait-based approach; yet, few studies have sought means to test for general patterns (Ackerly & Cornwell 2007; Ingram & Shurin 2009; Herben & Goldberg 2014).

The expected distribution of a given trait within a local assemblage will depend on the relative strength of abiotic and biotic pressures, as well as rates of productivity, disturbance and dispersal (Grime 2006; Chase & Myers 2011). Under environmentally stressful conditions, traits highly correlated with habitat characteristics should be clustered in community trait space due to environmental filtering. For example the presence of fish with special respiration adaptations in hypoxic floodplain pools (Chapman & Chapman 1993) or plants with short stature and

low leaf area in wind-exposed alpine environments (Spasojevic & Suding 2012) both reflect filtering of traits in response to stressful conditions. If habitat is spatially heterogeneous over the scale of study or space is limiting, traits associated with habitat features may be more functionally diverse than expected at random due to niche-stabilizing mechanisms of assembly (e.g. limiting similarity). Difference in body shape/size between fishes inhabiting riffles or pools (Lamouroux, Poff & Angermeier 2002; Troia & Gido 2015) and differences in limb and body size of ground-dwelling or arboreal *Anolis* lizards (Losos 2009) provide two examples of trait variation associated with habitat partitioning. Limited food resources may cause traits that are strongly associated with resource acquisition to be more functionally diverse than expected at random. For example Neotropical fishes (Lujan, Winemiller & Armbruster 2012) and birds (Trisos, Petchey & Tobias 2014) show large variation in mouth and beak morphology that likely reflect niche partitioning through alternative foraging strategies. Determining the conditions under which certain traits are most likely to respond to a given mechanism will be a critical step in developing a general theory of community assembly.

Progress towards understanding differential response of traits across niche axes will require novel approaches to test hypotheses about the expected distribution of trait values within local assemblages and how these compare to distributions under random assembly processes. Pioneering work on fish ecomorphology used stomach contents analysis to inform trait-based approaches (Gatz 1979), and several authors have explored relationships between stable isotope signatures of consumers and their functional traits (Lujan, German & Winemiller 2011; Domínguez *et al*. 2012; Gibb *et al*. 2015; Pool *et al*. 2016). Few studies, however, have used consumer isotopic signatures to interpret or predict how trait distributions within local assemblages compare to random expectations. Stable isotope signatures of consumers and food resources can be used to estimate consumer resource assimilation (Peterson & Fry 1987; Phillips *et al*. 2014), and relative positions in isotope bi-plot space can serve as proxies for relative positions in community trophic space, with variation in N isotope ratios reflecting differences in vertical trophic position and variation in C isotope ratios reflecting differences in the primary production sources assimilated (Layman *et al*. 2007; Perkins *et al*. 2014). Correlations between functional traits and isotopic signatures, combined with detailed knowledge of trait functions, may aid in determining a trait's association with trophic ecology and provide a basis for testing predictions of differential trait response. For example Ingram & Shurin (2009) used stable isotope ratios of nitrogen to show that traits related to trophic position in Pacific rockfishes have more evenly spaced values than expected at random, suggesting competition for resources has led to niche segregation in these assemblages. Similar approaches could provide insight into the complex patterns that occur when assembly mechanisms promoting

functional divergence interact with those promoting functional clustering, resulting in highly deterministic processes that produce community patterns indistinguishable from random assembly (Spasojevic & Suding 2012). If traits associated with resource acquisition play a stronger role in niche segregation, whereas other traits are more influential in how species respond to abiotic filters, the distributions of individual traits within local assemblages should reflect these differences regardless of the significance of overall community patterns (i.e. patterns derived from combinations of traits having diverse functions).

This study explores the relationship between trophic structure and the distributions of functional traits within assemblages of rapids-adapted fishes in the Xingu River, a major Amazon tributary. Studies in a range of stream and riverine habitats have found evidence of trophic niche partitioning in fishes (Gatz 1981; Ross 1986; Lujan, Winemiller & Armbruster 2012; Montaña, Winemiller & Sutton 2014), and there is strong evidence of convergence in the morphology of rheophilic fishes globally (Lamouroux, Poff & Angermeier 2002; Lujan & Conway 2015). This suggests that fish communities in rapids may experience strong pressure from opposing assembly processes, wherein traits involved in swimming and habitat use tend to converge and feeding traits show divergent patterns (Gatz 1981; Ackerly & Cornwell 2007; Ingram & Shurin 2009). Specifically, we test whether traits that have stronger correlations with stable isotope signatures (assumed here to reflect trophic ecology) display greater dispersion relative to a null model of community assembly. We further explore how well stable isotope ratios predict functional diversity patterns and whether *a priori* groupings of traits according to functions related to trophic strategy or habitat use differ in their associations with isotopic patterns.

Materials and methods

DATA COLLECTION

Analyses were based on fish surveys conducted during the 2013 dry season along a 400-km stretch of the Xingu River, the largest clear-water tributary to the Amazon River (Fig. 1A). This section of the river includes a 130-km complex of rapids and anastomosing channels over bedrock, known as the Volta Grande, inhabited by an exceptionally high diversity of fishes well adapted for life in swift water (Fig. 1B). Fishes were collected by cast net and by hand while diving/snorkelling with the help of local fishermen. Ninety-two species were collected from 20 sites, each of which was surveyed with 60–80 min of fishing effort over an area of c. 100 m^2 encompassing shallow rapids and deep, swift-flowing channels over rocky substrates. Dry-season survey data were selected for analyses because previous work revealed both functional clustering and functional divergence within these communities depending on the metric used (Fitzgerald *et al.* 2017a), suggesting the potential for different traits to be responding to different assembly mechanisms.

Functional traits were measured for 37 of the most abundant rheophilic species in our samples for which isotopic data were also available (see Table S1 in Supporting Information). These species represent nine families and accounted for 72% of the total fish specimens captured. The exclusion of rare species from the analysis should not impact results because the null model used (see below) maintained species occurrence frequencies, resulting in rare species having relatively little effect on overall trends. Due to insufficient sample sizes for species in the genus *Crenicichla*, and their high interspecific morphological and isotopic similarity, one nominal and three un-described species were pooled together (*Crenicichla* spp.) for the purpose of this analysis. Forty-five morphological traits, such as body depth/width, fin length/height, gut length and gill raker length, were selected based on their well-understood effects on performance with regard to habitat use and feeding ecology (Gatz 1979; Winemiller 1991; Winemiller *et al.* 2015). A full list of traits and descriptions of measurements are available in Table S2. Measurements were made on 3–6 adult individuals per species; length-based measurements were expressed relative to standard length, body depth, or head length/depth as appropriate and averaged for each species (Winemiller 1991). Species mean trait values were log, *n*th root, or inverse transformed to improve normality (see Table S2), and were standardized to zero mean and unit variance prior to analysis.

Samples used for isotopic analysis were collected from muscle tissue of three to seven adult individuals per species (with the exception of *Sternarchorynchus* sp. for which only two samples were available). In most cases, these were the same individuals used for functional trait measurements. Samples were prepared following standard protocols (Arrington & Winemiller 2002) and analysed at the Center for Applied Isotope Studies' Stable Isotope Ecology Laboratory at the University of Georgia, Athens. Ratios of heavy to light isotopes of C and N were expressed relative to standards (Pee Dee Belemnite and atmospheric N$_2$ respectively) and reported in delta notation (δ^{13}C and δ^{15}N). δ^{13}C and δ^{15}N values were used as estimates of relative position in trophic space. Because samples were collected from similar habitats within the same region during the dry season, spatial and temporal variation in isotopic signatures should not bias estimates. Samples for each species were taken from multiple sites throughout the study reach and average values per species were used for all analyses. Because variation in lipid content between species can impact analysis of C isotope ratios, δ^{13}C values were corrected using the equation: δ^{13}C$_{normalized}$ = δ^{13}C$_{untreated}$ − 3·32 + 0·99 × C : N (Post *et al.* 2007). In addition, benthic algae are the dominant production source supporting most fishes in this river during the dry season (Zuluaga Gómez *et al.* 2016), minimizing variation in vertical positions of consumers in isotopic space due to assimilation of alternative sources with different δ^{15}N.

DATA ANALYSIS

Five metrics that measure different components of functional diversity were used in analyses (Aiba *et al.* 2013): range estimates overall diversity of trait values present, variance estimates trait dispersion relative to the mean (i.e. centre of functional space), mean nearest neighbour distance (MNND) estimates how close trait values are in functional space, standard deviation nearest neighbour distance (SDNND) estimates how evenly trait values are spaced, and SDNND/range (SDNNDr) estimates how evenly trait values are spaced relative to the diversity of trait values present. For each survey site, these five metrics were calculated separately for each of the 45 functional traits measured based on the species present at that site (e.g. range of gut lengths present in an assemblage). Calculations were based on Euclidean distances between trait values.

These observed values for the five functional diversity metrics were then compared to values calculated from a null model based on species presence/absence within sites, where the regional species pool was the 400 km stretch of river and local sites were 100 m^2 reaches. The null model maintained observed species occurrence
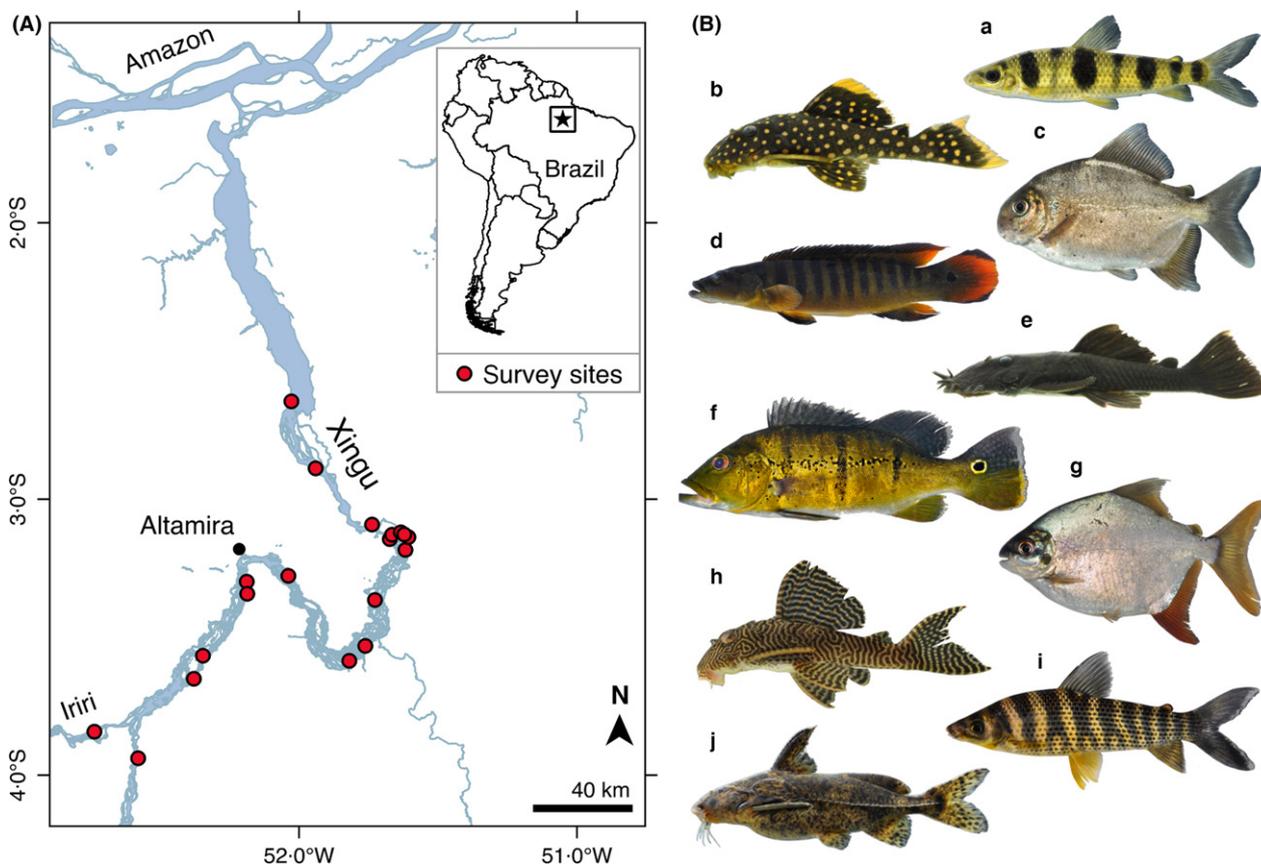
Fig. 1. Study system, showing (A) sampling locations and (B) several representative species. Each sampling location is a 100-m² reach and locations are separated by at least 1 km. Species shown are: a) *Leporinus maculatus* (Anostomidae), b) *Baryancistrus xanthellus* (Loricariidae), c) *Ossubtus xinguense* (Serrasalmidae), d) *Crenicichla* sp. (Cichlidae), e) *Ancistrus ranunculus* (Loricariidae), f) *Cichla melaniae* (Cichlidae), g) *Tometes kranponhah* (Serrasalmidae), h) *Hypancistrus* sp. (Loricariidae), i) *Leporinus fasciatus* (Anostomidae) and j) *Rhinodoras* sp. (Doradidae).

frequencies, as well as observed differences in species richness between sites. Randomizations followed the matrix-swap algorithm of Gotelli (2000) with a 1000 step burn-in and 500 step thinning parameter, implemented via the function RandomizeMatrix in the package picante (Kembel *et al.* 2010) using R version 3.2.2 for OS X (R Core Team 2015). Simulations were run 1000 times and functional diversity metrics were calculated for simulated communities as described above.

Standard effect size (SES) of each functional diversity metric was then calculated as (observed − mean_{simulated})/SD_{simulated} for each of the 45 traits within each of the 20 sites. SES measures how different the distribution of a trait within a local assemblage is from random expectation, with a positive SES value for range, variance and MNND indicating that the fish assemblage at that site has higher functional diversity than expected by chance for that trait. For example a positive SES range for gut length indicates that co-occurring species have a greater range of gut lengths than expected under purely random assembly. For SDNND and SDNNDr, a positive SES indicates that the fish assemblage at a given site is more unevenly distributed in functional space than expected by chance. Evenly spaced values are generally interpreted as evidence that species interactions play a strong role in structuring communities, assuming that intermediate trait values have been excluded through competition. Because SES reflects both the magnitude and direction of deterministic assembly processes, and facilitates comparison across studies, these values were used as a response variable in subsequent analyses. A key goal of trait-based ecology is to increase generality and predictability; focusing on

changes in SES rather than changes in functional diversity *per se* may provide a more effective means to understand the assembly process.

A linear modelling approach was then used to test for a relationship between a trait's divergence from random expectations (SES values) and a trait's correlation with the organism's relative position in assemblage trophic space (based on δ¹⁵N and δ¹³C). Spearman rank correlations were calculated between each trait and each isotopic element based on average values per species, providing a continuous measure of a trait's association with species position in isotopic space (δ¹⁵N and δ¹³C). Linear mixed effects models were used to control for the inherent correlations between functional diversity measures of different traits within the same local assemblage. SES values (i.e. trait divergence from random expectations) were included as response variables, absolute values of spearman rank correlations of each trait with δ¹⁵N and δ¹³C were considered fixed effects and site was included as a random intercept. Models were fit by restricted maximum likelihood using the package LME4 and parametric bootstrapped confidence intervals were used to assess significance of fixed effects (Bates *et al.* 2015). Constrained and marginal R^2 values were calculated following Nakagawa & Schielzeth (2013) using the function sem.-model.fits in the package PIECEWISESEM (Lefcheck 2015).

To test whether isotopic signatures explain a significant amount of the morphological variation present in the species pool, we used redundancy analysis (RDA) of standardized traits on standardized δ¹⁵N and δ¹³C values (i.e. the eigenvalue decomposition of the correlation matrix). RDA was performed using the function rda in

the package VEGAN (Oksanen *et al.* 2015). Tests of significance of RDA and explanatory variables were performed by permutation using 1000 iterations; tests of explanatory variables followed the marginal method of Legendre, Oksanen & ter Braak (2011). Constrained and residual ordinations were used to visualize niche trade-offs between species.

Finally, to facilitate comparison with previous studies that used trait categories rather than a correlation-based approach (e.g. Winemiller *et al.* 2015; Fitzgerald *et al.* 2017a), traits were divided *a priori* into two niche dimensions *sensu* Winemiller *et al.* (2015). Nineteen body and fin measurements that influence swimming performance and habitat use comprised the 'Habitat' category. Twenty-six traits that influence feeding performance, such as mouth width, gut length and tooth shape, comprised the 'Trophic' category (see Table S2 for full list). Because Trophic traits affect both foraging behaviour as well as relative position in assemblage trophic space, all of these traits may not necessarily be highly correlated with $\delta^{15}N$ and $\delta^{13}C$. For example species proficient at extracting aquatic invertebrates from interstitial spaces in substrates and others capable of removing invertebrates from submerged macrophytes may occupy similar positions in assemblage trophic space, but would likely have different head and jaw morphologies. Two-tailed t-tests were used to test whether mean trait correlations with $\delta^{15}N$ and $\delta^{13}C$ differed between the *a priori* trait categories and box plots were used to visualize the distribution of correlations across categories.

Results

A significant relationship was found between a trait's deviation from null expectations (i.e. SES values) and its correlation with isotope values, although results differed slightly between functional diversity metrics and isotopic elements (Fig. 2 and Table 1). SES variance was positively related to trait correlation with $\delta^{15}N$, indicating that a trait's variance in local assemblages increased as correlation with vertical trophic position increased (i.e. more diverse trait values present). SES variance showed no relationship with trait correlation with $\delta^{13}C$. SES MNND was negatively related to trait correlations with both $\delta^{15}N$ and $\delta^{13}C$. Contrary to our predictions, it appears traits are more clustered within assemblage functional space as correlation with species relative position in isotopic space increases. SES values of both SDNND and SDNNDr displayed a negative relationship (i.e. increasingly even) with trait correlations with $\delta^{15}N$, whereas only SES was negatively related with trait correlations with $\delta^{13}C$. In other words, traits became more evenly spaced among co-occurring species as correlation with species relative position in isotopic space increased. SES range showed no relationship with trait correlations with $\delta^{15}N$ or $\delta^{13}C$. Overall, these results reveal that traits with higher correlation with $\delta^{15}N$ tended to have values that were more variable, more clustered together and more evenly spaced within local assemblages compared to traits with low correlation with $\delta^{15}N$. Similarly, traits with higher correlation with $\delta^{13}C$ tended to be more clustered and more evenly spaced among co-occurring species compared to traits with low correlation with $\delta^{13}C$. Marginal R^2 values were consistently low (Table 1), demonstrating that fixed effects accounted for a small portion of the variance explained by each model.

Isotopic signatures explained a significant portion of the functional variation present in the species pool based on the full RDA model ($F = 2\cdot19$, $P = 0\cdot02$; Fig. 3). Further tests of explanatory variables found a significant effect of $\delta^{15}N$ ($F = 3\cdot56$, $P = 0\cdot01$) and no effect of $\delta^{13}C$ ($F = 0\cdot81$, $P = 0\cdot56$). RDA1 was largely influenced by $\delta^{15}N$, whereas $\delta^{13}C$ was strongly correlated with RDA2. Variation along RDA1 was mainly associated with traits related to feeding strategy, such as gut length, snout length, mouth position and oral disc width. Body width (which influences stability and turning ability) also loaded heavily on RDA1. The portion of variation explained by isotopic signatures was low (adjusted $R^2 = 0\cdot062$), with RDA1 and RDA2 explaining only 11·6% of total variation (Fig. 3). A large amount of residual variation remained in the first two principal component axes (Fig. 3b), with clearly interpretable functional groupings based largely on traits related to habitat use and swimming ability.

There was no significant difference between *a priori* trait categories based on comparisons of mean trait correlation with $\delta^{15}N$ (mean$_{\text{Habitat}}$ = 0·240, mean$_{\text{Trophic}}$ = 0·280, $t = -0\cdot82$, $P = 0\cdot21$) or $\delta^{13}C$ (mean$_{\text{Habitat}}$ = 0·142, mean$_{\text{Trophic}}$ = 0·160, $t = -0\cdot49$, $P = 0\cdot31$). While many of the highest correlations with $\delta^{15}N$ and $\delta^{13}C$ were for traits related to feeding (e.g. gut length, snout length, mouth width, tooth shape), not all traits in the Trophic category were highly correlated with isotopic values (Fig. 4; Table S3). Some traits in the Habitat category also had high correlations with trophic position (e.g. body width, caudal peduncle length and anal fin length). In general, trait correlations with $\delta^{15}N$ tended to be higher than those with $\delta^{13}C$.

Discussion

Support was found for our prediction that functional traits with higher correlation with species relative position in isotopic space tend to be more diverse and evenly distributed relative to other traits within local assemblages. The positive relationship found for SES variance and negative relationships found for SES of SDNND and SDNNDr (Fig. 2) suggest that traits associated with trophic ecology tend to diverge within local assemblages relative to other traits, and may be responding more to mechanisms promoting stabilizing niche differences (e.g. limiting similarity). The relatively large differences between conditional R^2 and marginal R^2 for some metrics (Table 1) indicate that species identity within local assemblages impacts these relationships, with the presence/absence of functionally distinct species (e.g. the nocturnal, electric fish *Sternarchorhynchus* sp. Apteronotidae) driving differences between sites. The negative relationship found for SES MNND did not match predictions, revealing that traits associated with trophic position also tend to be more clustered around specific values compared to other traits.

These results suggest that while species within local assemblages tend to segregate niches based on aspects of
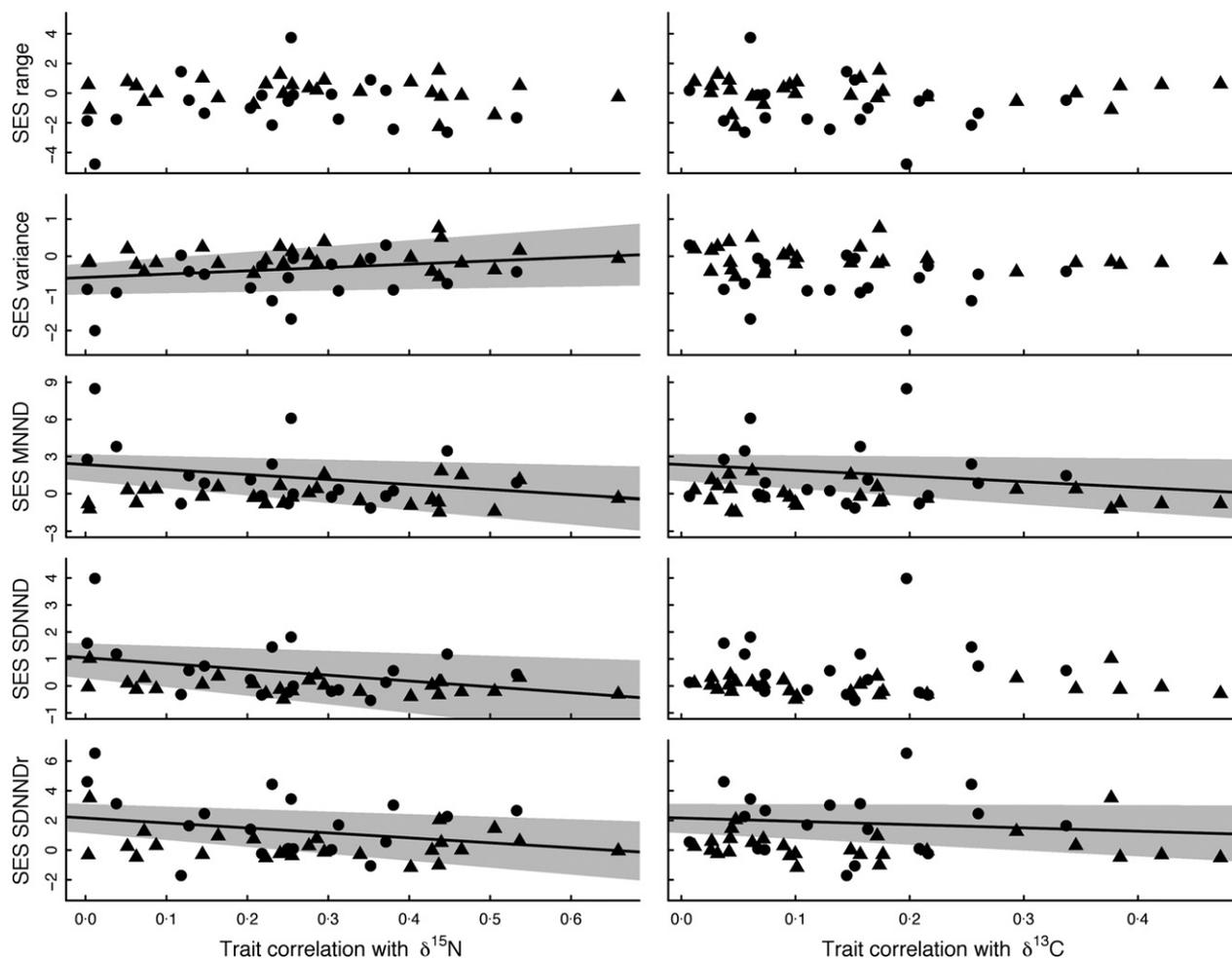
Fig. 2. Relationship between standard effect size (SES) and absolute value of spearman rank correlation with isotopic ratios of N (δ^{15}N, left panel) and C (δ^{13}C, right panel) for 45 morphological traits. Points represent mean SES values across 20 sites and are coded by trait category: Habitat (circles) and Trophic (triangles) for reference only (see Materials and methods). Black lines show mean trend for significant fixed effects of trait correlation with δ^{15}N and δ^{13}C based on linear mixed effects models with site included as a random intercept (parameter estimates provided in Table 1; fixed effects displayed separately for visualization purposes). Gray shaded regions represent 95% confidence intervals based on parametric bootstrapping. Abbreviations are for mean nearest neighbour distance (MNND), standard deviation of nearest neighbour distance (SDNND), and SDNND/range (SDNNDr).

trophic ecology, they remain highly clustered around some key trophic strategies. This apparent inconsistency may reflect niche diversification around certain adaptive peaks related to trophic ecology, such as benthic grazer and mid-water carnivore strategies. Rapids within the Xingu River support a high diversity of fishes in the family Loricariidae, many of which are common. While loricariids display considerable variation in trophic traits and isotopic values, their general body form is specialized for benthic feeding and many species occupy low trophic positions indicative of algivory and detritivory. Although some loricariids (e.g. *Scobinancistrus pariolispos* and *Leporacanthicus heterodon*) possess fewer and more robust teeth compared to algivorous species and displayed elevated δ^{15}N levels associated with partially insectivorous diets (Lujan, Winemiller & Armbruster 2012), those species occurred at fewer sites and had little influence on overall patterns. Compared to species in the remaining eight families analysed, loricariids

have gut lengths, snout lengths and mouth positions (traits highly correlated with isotopic ratios) that are tightly clustered in a distinct region of functional trait space. This results in a large group of common species with trait values that tend to be widely divergent from trait means (higher SES variance), evenly spaced from each other (lower SES SDNND/SDNNDr), but clustered together (lower SES MNND) due to shared ancestry and shared reliance on periphyton, detritus and other benthic resources. Clustering of these traits is also seen in families that tend to occupy higher trophic levels, such as the Cichlidae and Anostomidae, further driving the negative relationship found for SES MNND, and causing trait correlations with δ^{15}N to be generally higher than those with δ^{13}C. Despite the clustering of traits around the benthic grazer and mid-water carnivore strategies, traits more strongly correlated with δ^{15}N and δ^{13}C were more evenly spaced, suggesting that mechanisms promoting niche

Table 1. Results of linear mixed effects models for standard effect size (SES) of functional diversity measures

| Response | Trait correlation with $\delta^{15}N$ | | Trait correlation with $\delta^{13}C$ | | R^2_M | R^2_C |
	Estimate (SE)	95% CI	Estimate (SE)	95% CI		
SES range	0·36 (1·19)	(−2·09, 2·58)	−0·21 (1·64)	(−3·58, 3·09)	<0·01	0·06
SES variance	**0·89 (0·33)**	**(0·28, 1·53)**	0·21 (0·39)	(−0·61, 1·09)	0·01	0·23
SES MNND	**−4·03 (1·15)**	**(−6·35, −1·87)**	**−4·61 (1·59)**	**(−7·71, −1·79)**	0·01	0·07
SES SDNND	**−2·16 (0·62)**	**(−3·41, −0·98)**	−1·35 (0·85)	(−3·05, 0·25)	0·01	0·11
SES SDNNDr	**−3·34 (0·80)**	**(−4·79, −1·74)**	**−2·21 (1·10)**	**(−4·37, −0·04)**	0·02	0·24

Models fit by restricted maximum likelihood using trait correlations with $\delta^{13}C$ and $\delta^{15}N$ as fixed effects and site as a random intercept. Bold text indicates significant fixed effects based on parametric bootstrapped 95% confidence intervals (CI). Marginal (M) and conditional (C) R^2 values calculated following Nakagawa & Schielzeth (2013). Trend lines for significant effects are displayed in Fig. 2.
MNND, mean nearest neighbour distance; SDNND, standard deviation of nearest neighbour distance; SDNNDr, standard deviation of nearest neighbour distance/range.
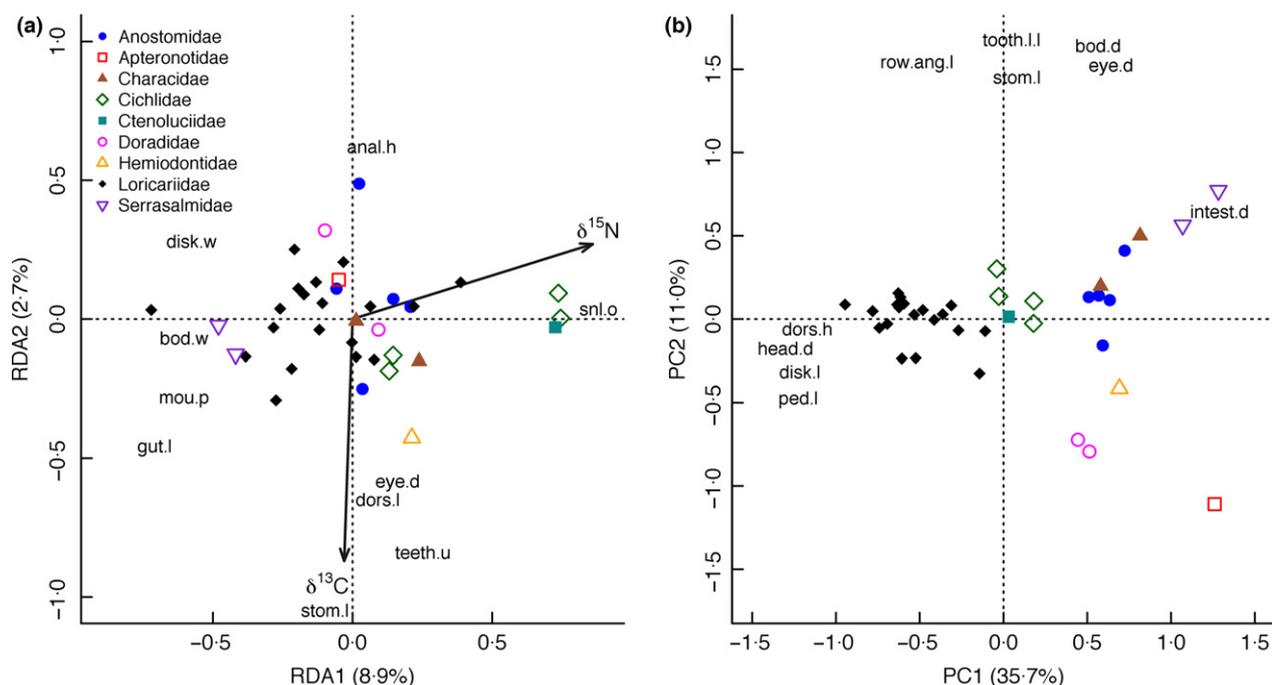

Fig. 3. Redundancy analysis of standardized morphological traits on standardized isotope ratios ($\delta^{15}N$ and $\delta^{13}C$). Points are mean values for each species and symbols represent different families. Only traits with the 10 highest factor loadings are presented for clarity; trait abbreviations represent the apices of vectors originating from the origin. Arrows represent explanatory variables. (a) Shows results of the constrained analysis plotted using the matrix of fitted values projected on the explanatory variables $\delta^{15}N$ and $\delta^{13}C$. (b) Shows residual variation not accounted for by the RDA model. Trait abbreviations and descriptions are provided in Table S2 in Supporting Information.

differentiation may have a stronger influence on trophic traits around these adaptive peaks. Consistent with this interpretation, previous studies of assembly patterns in loricariids and cichlids have suggested that these families partition niches along trophic dimensions (Lujan, Winemiller & Armbruster 2012; Montaña, Winemiller & Sutton 2014); however, the additional tendency towards functional clustering found in this study highlights how patterns obtained for specific taxonomic groups may differ from assembly patterns found within the broader community.

Our analysis was designed to reveal relative differences in trait dispersion; it did not directly test whether overall assembly patterns differ from those expected at random.

Physiological and evolutionary trade-offs may link traits responding to mechanisms promoting functional divergence (e.g. limiting similarity) with traits responding to even stronger mechanisms promoting functional clustering (e.g. abiotic filtering, competitive hierarchies), masking the expected patterns of the former. For instance despite the trend towards more even spacing between trait values with increasing trait correlation with $\delta^{15}N$, most traits had SES values close to zero and patterns were not distinguishable from random. Additional functional trade-offs exist that are not reflected by variation in trophic ecology (Fig. 3b), and species occurrence may be strongly influenced by mechanisms involving traits associated with other niche
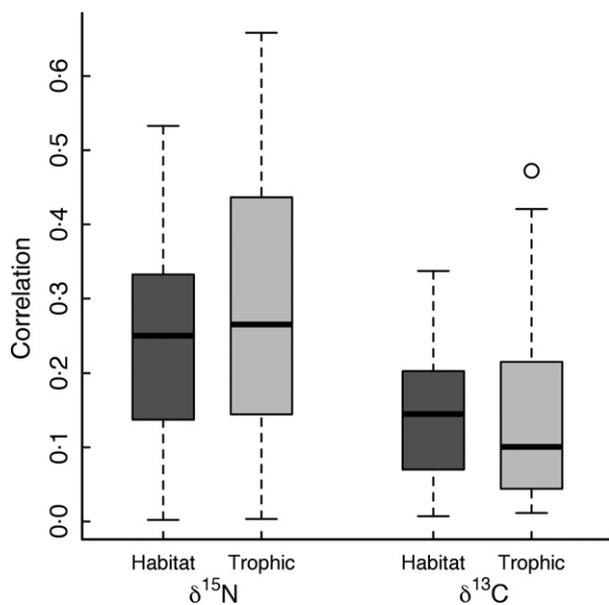
Fig. 4. Distributions of absolute value of spearman rank correlations between 45 morphological traits and isotopic ratios ($\delta^{15}N$ and $\delta^{13}C$) by trait category (Habitat and Trophic). For each elemental isotopic ratio, mean values between categories are not significantly different based on a two-tailed *t*-test.

dimensions (e.g. habitat use in response to environmental gradients), or along multiple dimensions simultaneously (Kraft, Godoy & Levine 2015). Indeed, a recent study by Pool *et al.* (2016) found that communities with similar functional characteristics often contribute to distinct food web structures, a result consistent with low coefficients of determination we obtained for both the linear models and RDA. Nevertheless, correlations between functional traits and stable isotope ratios were able to differentiate patterns of trait dispersion after a more general multivariate significance test had been performed (Fitzgerald *et al.* 2017a), providing further insight into patterns derived from multiple assembly mechanisms.

Because the goal of our study was to test trends based on *a priori* predictions rather than build predictive models, the low amount of functional variation explained by isotopic data does not impact interpretations. Our analysis included a large number of traits, many of which could affect trophic ecology in multiple ways; it is therefore not surprising that $\delta^{15}N$ and $\delta^{13}C$ explained low fractions of total functional variation in the RDA. Moreover, species distributions and co-occurrence are affected by trade-offs involving multiple niche dimensions, including habitat requirements, life-history strategies, trophic ecology, metabolic strategies and defence tactics (Winemiller *et al.* 2015). Because relative position in isotopic space captures trade-offs based on a single dimension, a great deal of unexplained variation would be expected. However, trade-offs associated with additional niche dimensions could be incorporated using similar methods, yielding more insights about the assembly process. For example variation along major environmental gradients (β-niche; Ackerly &

Cornwell 2007) could be incorporated through use of the 4th corner problem, RQL analysis or similar methods designed to correlate traits with environmental characteristics (Dolédec *et al.* 1996; Dray & Legendre 2008; Brind'Amour *et al.* 2011). Although data requirements are higher, this approach would be particularly useful for communities thought to partition niches based on habitat use and would complement analyses using stable isotope ratios or alternative measures of ecological performance.

Trait combinations can affect ecological performance, and trait interactions probably influenced correlations with isotopic signatures. Some even have argued that ecological and evolutionary trade-offs among traits bias analyses based on patterns of individual traits (Verberk, van Noordwijk & Hildrew 2013). In our study, the lack of significant difference between trait correlations with isotopic signatures for the Trophic and Habitat categories (Fig. 4) emphasizes the importance of identifying trait functions and trait interactions. Although certain Habitat traits (e.g. caudal peduncle length, anal fin length, body width) were highly correlated with $\delta^{15}N$, it is unlikely these traits influence feeding directly. The long caudal peduncles, fin lengths and dorso-ventrally compressed bodies of benthivorous loricariids compared to the relatively short caudal peduncles and narrow body widths of many omnivorous/carnivorous species of other families (e.g. *Teleocichla* sp., *Crenicichla* spp., *Boulengerella cuvieri*) drive this strong correlation. The dorso-ventrally compressed bodies and large fins of benthic fishes, and the fusiform shape and slender bodies of mid-water species influence hydrodynamics in swift water (Lujan & Conway 2015); however, because many of these species also have divergent trophic ecology, these traits were highly correlated with $\delta^{15}N$. Clearly, certain combinations of traits interact to affect species ecological performance, but these trait interactions were reflected in the correlations with isotopic signatures and should not bias results. Understanding of functional roles combined with an objective measure of association between traits and certain niche dimensions should allow us to identify differences in trait response along those niche dimensions, despite the inherent trade-offs and correlations between traits.

Our approach provides an effective means to tease apart the influence of multiple assembly mechanisms. Importantly, the expected relationship between trait dispersion and trait association with trophic structure will depend on the relative strength of the various mechanisms involved. For example no significant relationships were found between SES and trait correlations with $\delta^{15}N$ when analyses were repeated using wet-season data for the same fish assemblages (see Appendix S1). In many tropical rivers, expansion of aquatic habitat during the wet season leads to lower fish densities and an expected decrease in the strength of species interactions (Winemiller *et al.* 2014). Without the strong influence of species interactions to oppose the effects of abiotic filtering (i.e. trait divergence vs. trait similarity respectively), we would not expect a

significant relationship between trait dispersion and trait association with trophic structure. Indeed, previous research on Xingu fishes found that functional diversity of wet-season assemblages was significantly lower than null expectations, suggesting that processes promoting functional similarity (e.g. abiotic filtering) strongly influence these communities along multiple niche axes during the high-water period (Fitzgerald *et al.* 2017a).

Our analysis revealed that traits most strongly associated with species relative positions in isotopic space tend to be more functionally diverse with more even spacing among co-occurring species compared to other traits, which supports the idea that traits associated with different niche dimensions respond differently to various assembly mechanisms (Ingram & Shurin 2009; Herben & Goldberg 2014). The many studies reporting evidence of trophic niche partitioning in fishes suggest that this might be a general trend (Gatz 1981; Ross 1986; Ingram & Shurin 2009; Lujan, Winemiller & Armbruster 2012; Montaña, Winemiller & Sutton 2014). Additional studies are needed under a variety of hydrologic regimes and river sizes to test the generality of this pattern and how it relates to patterns and processes involving other niche dimensions (e.g. habitat use, life-history strategies, metabolic strategies). Analysis of individual traits or sets of functionally interrelated traits should improve understanding of the assembly process (Spasojevic & Suding 2012; Herben & Goldberg 2014; Trisos, Petchey & Tobias 2014; Winemiller *et al.* 2015); however, this approach may be complicated by inter-correlated traits and the possibility that a single trait affects multiple niche dimensions. The approach presented here offers a way to assess trait associations with particular niche dimensions in order to estimate trait response to alternative assembly processes. Methods that increase our ability to interpret complex assembly patterns will be critical to developing a general theory of trait-based community assembly.

Authors' contributions

D.B.F. designed the study, performed analyses and wrote the initial draft. M.H.S.P. and L.M.S. identified species. All authors collected data and contributed substantially to subsequent drafts.

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Data accessibility

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.67140 (Fitzgerald *et al.* 2017b).

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Supporting Information

Details of electronic Supporting Information are provided below.

Appendix S1. Results for wet-season assemblages.
Table S1. List of specimens used in analyses.
Table S2. Functional traits measured.
Table S3. Functional trait correlations with stable isotope ratios.
Table S4. Results of linear mixed effects models for standard effect size (SES) of functional diversity measures in wet-season assemblages.